EXPERIMENTAL STUDIES ON THE HISTOGENESIS OF MAMMARY TUMORS AND SEXUAL HORMONES

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EXPERIMENTAL STUDIES ON THE HISTOGENESIS OF MAMMARY TUMORS AND SEXUAL HORMONES

by

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I. INTRODUCTION

Recently, the number of patients with neoplastic diseases of the breast, especially, mastopathy (chronic cystic mastitis) have markedly increased in this country. Statistically, the death rate of mammary cancer in Japan has increased in recent years, but it still remained low compared with other countries—SEGI (1950). It seems probably that these differences in the incidence of mammary cancer in different populations depend especially upon the reproductive histories in each race.

Investigating thoroughly the condition of life of the recent patients with

mammary tumor especially mastopathy in our country Prof. Dr. AOYAGI has noticed clinically that those who are widows or have undergone spontaneous or artificial abortion, contraception and abnormal nursing showed a higher percentage than those who have led normal sexual life. MASUDA and others of our laboratory have recently measured the amount of estrogens and 17-ketosteroids in these patient's urine, and indicated the existence of sexhormonal imbalance due to relative hypoandrogenismus. From the results of the statistical and clinical investigations with regard to the striking increase of the neoplastic disease of the breast in Japan, it is very interesting to consider that the reproductive histories of the Japanese women in recent years have become to resemble those of the westerners.

In 1946 KOGURE has reported the relationship between mastopathy-like-changes and repeated abnormal nursing in mice. Recently, experimental investigations on neoplastic disease of the mammary gland in mice have progressed remarkably. According to the present concept the development of mammary cancer in mice is dependent upon 3 main factors: the genetic constitution of the animal, the hormonal stimulation to which the mammae are subjected, and the presence of a virus-like agent transferred to the offspring through the milk of the nursing female. It seems that the development of the precancerous hyperplasia of the mammary gland in mice must be also dependent upon these 3 main factors. This hypothesis was ascertained by the detailed experiments of BITTNER (1946). However, the virus in mammary tumors on many inbred and hybrid mice has not been recognized. Moreover, the pathogenesis of mammary cancer in human being is yet unknown and no virus-like agent has been recognized.

These studies are, then, an attempt to bring into closer association the problems connected with mammary tumors in human being and in the hybrid mouse. In particular, they are concerned with the comparison between the morphologic structure of supposedly precancerous lesions in the mouse and in man and with the possibilities of comparable endocrine disturbances underlying each. From this point of view, a relationship between the neoplastic disease of the breast and both the influences of nursing failure and the hormonal stimulation is observed in mice.

II. MATERIALS AND METHODS

1. Animals

All the mice used were females of the hybrids from Gifu in Japan. Inbred mice were not used because of the difficulty in procuring large numbers at the same time in this country. On the other hand, no milk-agent has been recognized both in human being and in the hybrid mouse, therefore it was thought that the similar results in clinical observations might be accounted for by the statistics of this hybrid mice. For food rice, vegetable, fish meal, cod liver oil and salt were given. All the mice were treated under same condition.

2. Experimental procedure

The groups of mice used in this study may be summarized as follows: Lactation groups (269 cases).

1) Interrupted breeding group (160 cases).
2) Non-breeding group (21 cases).
3) Normal breeding group (88 cases).

Virgin female group (110 cases).

Hormon groups (102 cases).

1) Estrogen group (62 cases).
2) Androgen group (40 cases).

For the experiments on lactation the method described by KOGURE (1946) was employed.

The young female mice were nursed with the young male mice, and when the females became pregnant, they were delivered in the isolated pens. Thirty days after parturition, the females were again returned to the breeding pens. All the experimental mice with repeated pregnancies were divided into three groups. Each group was treated with a different way of nursing. In one (I group), the females were permitted to nurse their progeny for approximately 5 days after which their progeny removed: in another (II group), the females were not permitted to nurse and their progeny were removed immediately after birth as soon as they were discovered, which was usually within a few hours: in the third (III group), the females were permitted to nurse their progeny for 30 days after which they were returned to the breeding pen. Moreover, the animals of the third group were divided into 2 groups, those which had from 6 to 13 litters and those having within 5 litters.

The hormon groups consisted of estrogen group and androgen group. The hormones, estradiol pellet and testosterone propionate pellet, were administered to the females of a 30 days old. In the estrogen group the females received insertions of 1 25mg of estradiol pellet every 50 days for 3 times. In the androgen group the females received insertions of 6 25mg every 50 days for 3 times.

In order to obtain the highest changes in the mammary gland, the mice were killed at the last moment of their natural death varying from 90 to 745 days.

3. Technical procedure

There are usually 5 pairs of glands in the mouse, arranged symmetrically along the ventral and lateral surfaces of the body. Three in the thoracic and 2 in the abdomino-inguinal region. During lactation, the whole subcutaneous area from behind the ears to the base of the tail, except for a small strip in the midline of the back and abdomen, may be covered by milk engorged mammary tissue, and they are found most frequently in areas where the bulk of the mammary tissue is greatest.

The mammary gland in mice was studied histologically by means of both the whole mount and section technics. The whole mounts of the mammary glands were prepared according to the method described by COWIE & FOLLEY (1947) and FLUX (1949).

The skins was slit up the dorsum and carefully removed so that the mammary glands left intact and adherent to the removed skin. The skins were pinned flat on wooden boards with minimum stretching and fixed in BOUIN's fluid for 24 hours, washed in running water for 24 hours stained in MAYER's haemalum for 24 hours and decolorized in 80% acid alcohol. When the skins were sufficiently clear, they were

placed over a light in a Petri dish containing 70% alcohol, the nipples were located, and the skin was peeled off in small strips from the part of the tela subcutarea supporting the mammary glands. The skins were returned to the stain for a further 24 hours, decolorized in acid alcohol and blued in tap water. Individual glands were dissected out with fine pointed forceps, dehydrated in absolute alcohol and mounted in Canada balsam from xylol. After staining, all the glands were inspected, and the interesting histological changes were taken in microphotographs, then they were embedded in paraffin, sectioned at 5 micra.

The whole mounts appear to us almost essential. The mouse mammary gland is spreads out over a wide area but lies almost in a single plane. Microscopic sections cutting across this plane show only a few ducts and give a poor conception of the gland as a whole.

III. NORMAL DEVELOPMENT OF THE MAMMARY GLAND IN FEMALE MICE

1. Observations

1) Normal development

The mammary glands at birth consist of only a few ducts which are straight unbranched extensions. The mammary ducts of a 15-day-old mouse showed prominent end buds which stained deeply with haemalum (Fig. 3). From birth until the approach of puberty, the rudiments of the mammary glands of female mice continue to grow slowly. The ducts of a 50-day-old mouse grow peripherally from enlarged proliferating end buds, and new branches develop from buds forming along the walls of the existing ducts. The development progresses steadily up to the age of 50 days. Between the ages of 42 and 56 days female mice reach sexual maturity. At the time of the first ovulation an acceleration of growth occurs and the ducts develop many side branches. About 90-day-old, the development of the mammary glands is completed (Fig. 4). Since then, the glands change very little other than the slight cyclic hypertrophy of the ducts with each estrus or early post-estrus period. Microscopically only a few ducts are shown in the adipose tissue (Fig. 5). The large ducts are lined with two or more layers of cuboidal epithelial cells, whereas, the small ducts and the end buds are lined with imcomplete two layers of epithelial cells. The imcomplete two layers consist of a lining of cuboidal epithelial cells and the extensor layer of the socalled "Myoepithelialen" epithelial cells which are less than the numbers of the lining cells. (Figs. 27 and 32). The epithelial cells of the small ducts are somewhat taller than those in the large ducts.

The normal mammary glands have scarecely any fibrous tissues, which is an important point in diagnosis of the pathologic changes of the mammary glands (Figs. 6, 7 and 8).

2) Pregnancy

The young mice are born on the 21st day of gestation. At the 7th day of pregnancy the ducts increase in length and many side branches are formed (Figs. 9, 10 and 11). During this period many end buds are present at the distal ends

of the ducts. By further development all the end buds and the terminal twigs dilate and unfold into alveoli. At the 20th day of pregnancy the glandular system is well developed (Figs. 12, 13 and 14). Only at the distal ends away from the nipple are the ducts still growing. Further development consists of hypertrophy and enlargement of the lumens. The ducts end in terminal alveoli which at this stage begin to show secretory activity. Namely, the developing glandular system occupies more and more space and the adipose tissue is rapidly diminishing.

3) Lactation

The first few days after parturition all the mammary glands are in full function. Microscopical sections show that the ducts are dilated and filled with milk (Figs. 15 and 16). The glandular parenchyma is in excess and the adipose cells only fill in the space left by it. The number of mitotic figures is definitely noticeable at the 4th to 5th day after parturition and reaches its peak at about 12th day. The whole mount preparation in this stage stained entirely deeply with haemalum. Since then, these glands will undergo a certain degree of regression.

4) Regression after lactation

Following weaning, resorption of the secretion and collapse of the alveoli occur (Figs. 17, 18, 19, 20, 21, 22 and 23). Regression is a reversed process of the changes that take place during pregnancy. Microscopic sections show that 24 hours after ablactation the alveoli and the ducts become distended by the accumulation of the milk, which gradually diminishes and most of the alveoli and the terminal ducts begin to collapse. They lose their alveolar structure and form irregular groups of epithelial cells which are surrounded by the parenchyma with fibrous connective tissue. The amount of the adipose tissue increases proportionately with the regression of these parenchyma. Regression is usually complete about 14 to 30 days after nursing stops. There are no alveoli. The glands can be now considered a "resting" gland. The process of regression is not very uniform. Considerable variation exists between the glands of the same animal, depending on whether the nipple was suckled or not up to the time of weaning.

2. Discussion

The normal development of the mammary glands of the mice has been extensively discussed by TURNER & GOMEZ (1933), FEKETE (1938), GARDNER & STRONG (1935), DALTON (1945) and FLUX (1954). FEKETE described that the development progresses steadily up to the ages of 6 weeks. His account agrees in general with that of the several author's. In our observations, the changes occured in the mice corresponded to those of the other workers. Namely, at the age of 50 days, the duct system began to grow rapidly and the mammary ducts were completed about the age of 90 days.

GARDNER & STRONG (1935) gave an excellent account of the changes in the mammary gland of the mice during the estrus cycle. "During estrus and early postestrus, the mammary ducts were slightly distended" This observation verified those of other investigators—WIESER (1934) and FLUX (1954).

During the period of regression the duct system of the breast in human being

is lined with two layers of cells, and in the period of pregnancy many round elongations protrude from the walls and the ends of the ducts. By further development all the buds unfold into alveoli which are lined with a single layer of cells. Accordingly, FUJISUE (1955) stated that the human glandular system during regression is consisted of socalled "lobulus tubulosus or lobulus nonlactans" which are fine ducts of double-layer cells grouped together. In the other hand, the glandular system during pregnancy and lactation is consisted of socalled "lobulus alveolaris or lobulus lactans" which are alveoli of single-layer cells grouped together. APOLANT (1906) stated that the smaller ducts and end buds of the mammary glands in mice were lined only with a single layer of cells. WIESER (1934) observed that the peripheral part of the ducts of the mammary gland in mature mice are consisted of two imperfect layers of cells. Our observations corresponded with the WIESER's report.

These findings presented some important suggestion on the analysis of the histological changes in neoplastic diseases of the breast; as the precancerous changes of the mammary glands in mice appeared usually in the peripheral parts of the ducts. It should be emphasized also that the early changes which lead eventually to the development of spontaneous carcinoma in mice affect essentially the peripheral portion of the mammary gland.

The normal mammary glands in mice have no lobulus during regression. TAMAGAWA (1927) and NISHIGAKI (1952) have observed that during the earlier stage of pregnancy the lobulus is consisted of fine ducts and during latter stage of pregnancy or lactation the lobulus is consisted of alveoli. In the present study we have included both the fine ducts and the alveoli in description of the lobulus.

TAMAGAWA (1927), HIRATA (1928), FEKETE (1938) and NISHIGAKI (1952) have reported on the mammary glands during the period of regression. The disappearance of the lobuli; retention of the fibrous connective tissue surrounding the parenchyma are in common, but the characteristic point in mice is that the increased connective tissue soon vanish and the adipose tissue appear to take its place. The greater part of the normal mammary tissue of most of the animals was either undergoing regression or remaining inactive. Sometimes, incomplete regression and persistence of secretory cells were observed. The gross structure of the normal glandular tissue of mice varied greatly, although each individual presented structually uniform glands. The variations observed were chiefly in the extent of regression. GARDNER (1939) have classified the glands into 5 groups on the basis of its degree of involution (Figs. 17, 18, 19, 20, 21, 22 and 23).

FUJISUE (1956) described the changes in the duct system of the mammary glands of female mice as the result of the effect of the estrogens. He concluded that the histological source and precancerous origin of mastopathy and the precancerous lesion may be considered to be in the duct II and duct III spheres. Our observations on the classification of the ductual system in mice agree in general with that of FUJISUE's. The epithelia of the duct II and the duct III have the same histological structure. It is probable that the same changes may occur during pregnancy (Fig. 1).

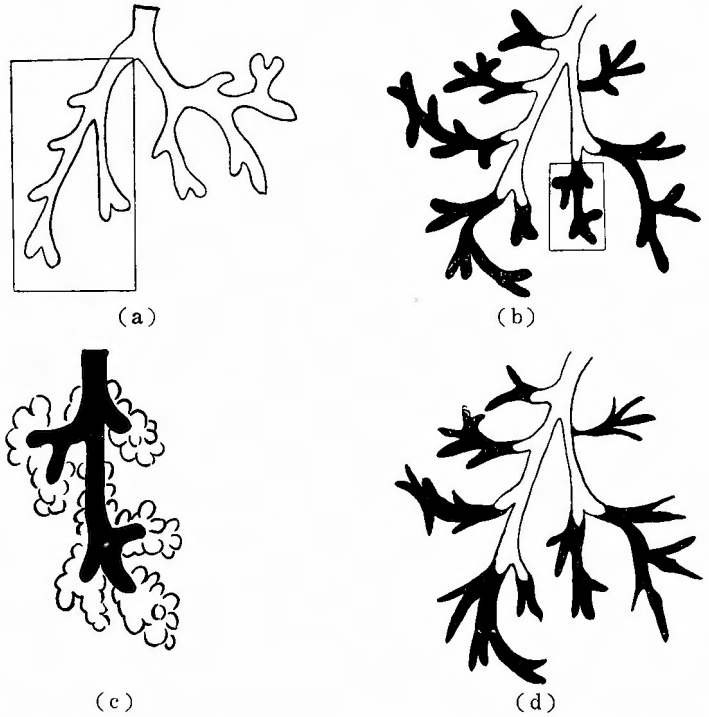

Fig. 1. Diagram of a development of the mammary ducts in mice. The dark ducts mark the position of the duct Ⅱ and duct Ⅲ.
a) Mammary duct of a 30 days old female mouse. b) Mammary duct of a 90 days old female mouse. c) Mammary duct on the 20th day of pregnancy. d) Mammary duct on a regression stage after lactation.

IV. PATHOLOGIC CHANGES OF THE MAMMARY GLAND IN MICE

1. Pathological histology and classification of mastopathy-like-changes in mice

The correlation of mammary gland structure with the occurrence of malignant growth had been clarified early in the study of mammary cancer in mice. The precancerous nature of localized areas was established by demonstrating transitional stages between them and frank cancer. The localized areas are also encountered in the mammary gland of mice that developed mammary cancer following prolonged treatment with estrogens. The relationship of these localized areas to mammary carcinogenesis was also emphasized by HAALAND (1911), GARDNER (1935), FEKETE (1936), TAYLOR (1940), HUSEBY & BITTNER (1946) and generally accepted. From the work of GIBSON (1930), WIESER (1934), BURROW (1936), MAC DONALD (1936), BONSER (1936), LACASSAGNE (1936), TAYLOR (1940), KOGURE (1946) and FUJISUE (1954) these localized areas in mice were named "zones of chronic cystic mastitis, oestrogenic mastopathy or mastopathy-like-changes" because of the morphologic similarity to some of the forms of human mastopathy.

Mastopathy in the human being shows a complicated histological picture. Therefore, neoplastic, inflammatory and regressions theories have been given to this disease. The normal mammary tissues are composed of groups of ducts and cellular

connective tissue. The pathologic structures of mastopathy, after all, can be summarized as the changes of both of these ducts and the connective tissue. The mammary gland may present regression of the mammary tissue, and at the same time there may also be present duct dilatation and hyperplasia of the epithelium and fibrosis and also the occurrence of "eosinophilic epithelium cyst" and round cell infiltration of the connective tissue.

Actually, these analogous histological changes of mastopathy in the human being may almost be recognized in the mouse with the exception of the changes of "diffuse fibromatosis" or "eosinophilic epithelium cyst".

These changes of the mammary glands in mice were divided into 3 types on abnormal lesion by HUSEBY & BITTNER (1946) and also it were divided into 3 types on mastopathy-like-changes in mice by FUJISUE (1954).

After comparable and considerable study on the whole mount preparations and the microscopic sections it seemed preferable to classify the lesions present in the material supplementing the classification of the HUSEBY and BITTNERS report under four principal groups.

I (1). Areas composed primarily of hyperplastic fine ducts in otherwise resting glands.

I (2). Areas composed of dilated fine ducts of the above.

II. Areas composed of extensive proliferation of relative fine ducts.

III. Areas composed of a few hyperplastic ducts surrounded by considerable inflammatory tissue reactions.

IV. Areas composed of cystic alveoli and dilated ducts surrounded by connective fibrous tissues.

Type I (1). The first type of glandular abnormity composed of the hyperplastic fine ducts from duct III and duct II. In whole mount preparation, nodules of denser epithelial tissues are present at random in otherwise resting glands (Figs. 24 and 25). The nodules are composed of diffuse overgrowth of fine ducts. These overgrowth mainly arose from a single area rather along the side of the duct III than the duct II. The histogenesis of these nodules showed that they must be reffered to the hyperplastic epithelial cells of mainly the duct III, and according to more classical histological nomenclature probably correspond to the intralobular ducts (better called "ductules" as DAWSON points out in mastopathy).

In microscopic sections these hyperplastic areas also showed proliferation of abnormal fine ducts (Figs. 26 and 27). The primary alteration in such a lesion was hyperplastic areas, with but slight to moderate increase of the supporting connective tissue and no appreciable evidence of inflammatory reaction. Many mitotic figures appeared among the hyperchromatic epithelial cells of these growths (Figs. 28 and 29).

These lesions were the most frequent of these 4 lesions, and from this numerical standpoint these lesions have been previously referred to an important precursor of mammary cancer in mice by APOLANT, HAALAND, GIBSON, GARDNER, FEKETE, TAYLOR, KOGURE, HUSEBY and FUJISUE. However, the areas were not always subjected to

precancerous changes except for the localized small part in these areas, the major parts of the hyperplastic areas transformed reversibly into a degenerated state with the physiological mammary involution in advanced age Nevertheless, a few small hyperplastic parts were not rarely encountered in these involuted areas. It seemed that such a hyperplastic area was indeed a source of malignant transformation of mastopathy like lesions in mice.

The areas of this type in mice bear a close resemblance to some histological findings which are named "Adenosis" "Type II in mastopathy (MASUDA)" and "Mastopathia simplex (TAKEDA)" in human mastopathy.

Type I (2). Areas composed of dilated fine ducts and their overgrowth become to form large nodules, namely the fine ducts of the Ist type (1) are often somewhat distended with inspissated secretion, their epithelium frequently exhibits appreciable mitotic activity, and the surrounding layer of collageneous connective tissue is somewhat thicker than is normally seen (Figs. 30, 31 and 32). Differentiation between large ducts (Type II) and the dilated fine ducts (Type I (2)) seems almost impossible without the aid of these whole mount preparation.

The areas of this type in mice, as well as the areas of Type II, bear a close resemblance to some histological findings which are named "cystic ductal type" "cystic diseases (NATHANSON)" and "Type III in mastopathy (MASUDA)" in human mastopathy.

Type II. The second type of glandular abnormity encountered is one of the ductular proliferation described previously by GARDNER (1942). However, this change occurred infrequently. It consists of multicresentric areas in which the extensive, proliferative mammary ducts (duct II and duct III) radiates from a relative small locus, so that the lesion simulates a many pointed star of which the radii, through often branched, are smooth as neither intralobular ducts nor alveoli develop along them. Although the changes occur much less frequently than the Ist type, some precancerous parts are also found in the center of such areas. (Figs. 33 and 34).

The areas of this type in mice as well as the type I (2), bear a close resemblance to some histological findings which are named "cystic disease" in human mastopathy (Fig. 35).

Type III. The third type of lesion appears to be inflammatory in nature, for in extensive lymphocytic infiltration there are less pronounced ductual hyperplasia than in the typical ductual nodules. The extent of the reaction varies widely from nodule to nodule; in some the glandular elements may slightly predominate whereas in others they are seen only with difficulty in whole mount preparations (Figs. 36 and 37). However, foci of lymphocytic infiltration without glandular hyperplasia are not to be included with this type of lesion. Though it has been described by HUSEBY & BITTNER (1946) that these lesions consist of glandular to squamous-type epithelium, often with a good deal of desquamation of keratinized material into distended alveolar lumina, in our material such change occurs infrequently.

We have felt that this type of nodules does not predispose to the spontaneous cancer of mice. It seems that the occurrence of this lesion suggests a genesis of the inflammatory nature in mastopathy, that was previously called "chronic cystic

mastitis (KÖNIG)"

Type IV. The areas of the fourth type of these lesions are composed of cystic alveoli and the dilated ducts are surrounded by the connective fibrous tissues. In the whole mount preparation an increase of the fibrous tissue is observable immediately beneath the dilated ducts (Fig. 38). Histological examination shows the ducts dilated to cystic proportions and filled with a little secretion. The lining cells are usually flattened and overlie a zone of connective tissue infiltrated with lymphocytes. Finally, the fibrous tissues increase comparatively in the mammary tissue (Fig. 39).

WIESER (1934) described that these fibrosis appeared in old mice and these changes bear a close resemblance to that of "Fibroadenoma intra et pericanaliculare" in the human being. The normal mammary glands in mice consist little of fibrous tissue. Therefore, it seems reasonable to classify the fibrosis of the mammary glands in mice as mastopathy-like-changes. In fact, definite proliferation of connective tissues had appeared always about the ducts in a few mice, and it may probably be explained on the basis of a reaction to chronic inflammation. The histologic picture of the common type of human mastopathy in which connective tissue proliferation leads to diffuse adenofibrosis was not reproduced by this means.

The areas of this type in mice bear a close resemblance to some histological findings which are named "Type IV in mastopathy (MASUDA)" and "cyst without epithelial hyperplasia (NATHANSON)" in the human being. We felt that these lesions do not predispose to the spontaneous cancer of mice.

"Apocrine metaplasia" comparable to that so frequently encountered in the human mastopathy was never observed in these mice. TAYLOR (1940) described that on account of basic anatomic differences between the human and the mouse mammary glands, it cannot be expected that morphologically identical lesions should occur. WIESER (1934) stated that the mouse mammary glands had anatomically no sweat gland epithelium.

Thus mastopathy-like-changes in mice are classified into four types. However, different mammary glands in the same animal are of different morphological types, and different areas of the same mammary gland occasionally vary in different types.

2. Histology and classification of mammary tumors in mice

The mammary tumors grow progressively and may reach enormous size; often additional tumors appear at others sites. The first to classify mammary tumors in mice was APOLANT (1906) and HAALAND (1911), CLOUDMANN (1941), AKAMATZU (1956) and others have made similar efforts. BORREL (1903) stated that the type of the mouse tumor was always the same and nearly always made up of a fine tubular structure, or coarse cylinders, or true epithelial buds in a loose connective tissue. AKAMATZU has classified the mouse tumors into four groups viz. ductal type, acinar type, the metaplastic type which is the result of metaplasis of the above two, and lastly sarcoma. Namely, the tumors arising from the ductal epithelium are separated in the classification from those arising from the acinar cells. However, it would be difficult to attempt to classify histologically whether these mammary tumors are originated from duct, or gland epithelium. DUNN has also stated that

it would be most difficult to make a definite classification of the histopathology of mammary tumors in mice. However, DUNN also emphasized that every tumor would not fit into the exact category and that different individuals probably would not arrive at the same classification for every tumor, especially if they might be looking at different sections.

The following groups have been listed by DUNN, with their synonyms:

Adenocarcinoma, Type A. (Typical mammary tumor, mammary adenocarcinoma, alveolar carcinoma). In these tumors there is predominance of the acinar structure (Figs. 40, 41, 42 and 43).

Adenocarcinoma, Type B. (Papillary cyst adenocarcinoma, intracanalicular adenocarcinoma, carcinoma simplex). There is no definite demarcation between tumors of Type A and B; differentiation is based primarily upon the amount of acinar structure (Figs. 44 and 45).

Adenocarcinoma, Type C. (Fibroadenoma, adenofibroma). These tumors are made up of many epithelial-lined cysts, with the lining closely invested by a layer of spindle cells.

Adenoacanthoma. (Keratinized mammary tumors, adenocancroid, adenosquamous-cacinoma, mammary tumor with squamous metaplasia (Fig. 46).

Carcinosarcoma. (Carcinoma wtih spindle cell formation. anaplastic carcinoma, mixed tumors).

Sarcoma of the mammary gland area (Mammary sarcoma).

Miscellaneous types, consisting of bizarre tumors.

The adenocarcinoma, Type A and B were observed more frequently in these materials, while adenoacanthoma was observed only a few, and other types of mammary tumors were not observed. Different tumors in the same animal were of different morphological types, and different areas of the same tumor occasionally varied in the arrangements assumed by the tumor cells. Variations in the same tumor complicate the problem of classification, therefore all the mammary tumors in this study were only called "mammary cancer" in a lump.

V. INFLUENCE OF NURSING DISTURBANCES ON THE MAMMARY GLAND IN MICE

1. Experimental results

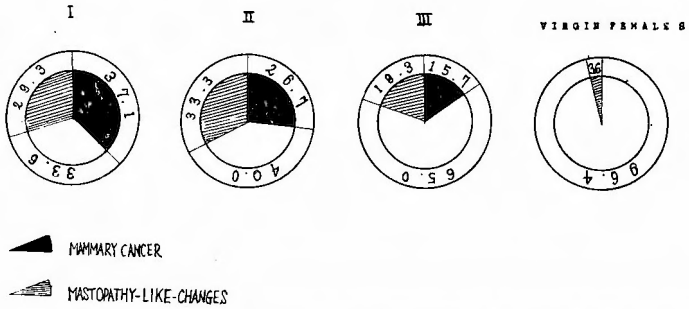

Fig. 2. Incidence of mastopathy-like-changes and mammary cancer in interrupted breeding (I), non-breeding (II), normal breeding (III) and virgin female mice.

Two hundred and sixty nine females are divided into 3 different nursing groups comprising 160, 21 and 88 animals, respectively.

The details of the data obtained from these experiments are summarized in Table 1 and illustrated in Fig. 2. The most variable gland in the same animal is indicated in the table for the histological changes of the mouse. On account of the existence of mammary cancer and mastopathy-like-changes in the same animal they are included as mammary cancer in the table. The numbers and the percentages of the mice pregnated more than twice are given on the right side of the table.

The percentages of mastopathy-like-changes and mammary cancer in the first

Table 1. Incidence of mastopathy-like-changes and mammary cancer in interrupted breeding (Ⅰ), non-breeding (Ⅱ) and normal breeding (Ⅲ) female mice.

Group (Number of mice)	Histological appearances of the mammary gland		Number of parturition 1	2	3	4	5	6	7	8	Total		Numbers and percentages of the mice pregnated more than twice
Ⅰst group (160)	−		13	9	9	9	8	1	1		50	37	47 (33.6%)
	Mastopathy-like changes	±	1	1	3	3	1	1		1	11	10	
		Type Ⅰ(1)	1	1	6	3	3	2			16	15	41 (29.3%)
		Type Ⅰ(2)		1			2		1		4	4	
		Type Ⅱ			3		3	1			7	7	
		Type Ⅲ			1						1	1	
		Type Ⅳ	4	1	2	2	2				11	7	
		Ⅰ……Ⅱ				3	1		1		5	5	
		Ⅰ…Ⅱ…Ⅲ	1				2				3	2	
	Mammary cancer			4	4	13	13	12	4	2	52	52	52(37.1%)
Ⅱnd group (21)	−		4		2	1	2				9	5	6 (40.0%)
	Mastopathy-like changes	±			1						1	1	
		Type Ⅰ(1)	2				1	1			4	2	5 (33.3%)
		Type Ⅰ(2)		1							1	1	
		Type Ⅱ											
		Type Ⅲ		1							1	1	
		Type Ⅳ				1					1	1	
		Ⅰ……Ⅱ											
	Mammary cancer			1		1	1	1			4	4	4(26.7%)
Ⅲrd group (88)	−		4	7	14	16	11	3			55	51	54 (65.0%)
	Mastopathy-like changes	±					3				3	3	
		Type Ⅰ(1)	1	1	1	1	2	2			8	7	16 (19.3%)
		Type Ⅰ(2)						1			1	1	
		Type Ⅱ			2	3					5	5	
		Type Ⅲ											
		Type Ⅳ		1	1						2	2	
		Ⅰ……Ⅱ							1		1	1	
	Mammary cancer				1	5	4	3			13	13	13(15.7%)

group of the females with 5-days nursing period were 29.3 per cent (41 out 140 mice) and 37.1 per cent (52 cases) respectively. The percentages of those in the second group of non-breeders were 33.3 per cent (5 out of 15 mice) and 26.7 per cent (4 cases), and those in the third group of the females which nursed their young for 30 days were 19.3 per cent (16 out of 83 mice) and 15.7 per cent (13 cases) respectively. Both the prevention of nursing and the non-breeding have effected on the incidence of mammary lesions, as compared with the normal breeding. Especially, the females which nursed their young for 5-days in the first group showed the highest incidence of mammary cancer.

Table 2. illustrate the mammary cancer with mastopathy-like-changes contained in Table I. The incidence of mammary cancer with mastopathy-like-changes of

Table 2. Correlation between mammary cancer and mastopathy-like-changes in lactation-group-mice.

Groups (Number of mice)	Mammary cancer with mastopathy-like-changes		Mammary cancer without mastopathy-like-changes	
	Differnt glands in the same mouse, Mastopathy (+)	Differnt glands in the same mouse, Mastopathy (−)	Different glands in the same mouse, Mastopathy (+)	Different glands in the same mouse, Mastopathy (−)
I st group (52)	28		24	
	17	11	11	13
II nd group (4)	1		3	
	1	0	2	1
III rd group (13)	4		9	
	3	1	3	6

the first group was 53.8 per cent (28 out of 52 mice), those of the second group was 25.0 per cent (1 out of 4 mice), and those of the third group was 30.7 per cent (4 out of 13 mice). The average of the three groups was 47.8 per cent (33 out of 69 mice). All the groups showed a high incidence, especially, the first group showed the highest incidence in these groups. Moreover, different glands in the same animal with mammary cancer without mastopathy-like-changes showed the high rate of incidence of mastopathy-like-changes.

Table 3. gives the location of 69 mammary cancer; 28.6 per cent were in the axilla region on the right side (A); 25.3 per cent in the axilla region on the left (B); 24.1 per cent in the inguinal region on the right (C); and about 22.0 per cent in the inguinal region on the left (D).

Table 3. Location of mammary cancer in lactation-group-mice.

Group \ Location	A	B	C	D
I	19	15	18	15
II	2	3	0	0
III	5	5	4	5
Totals	26	23	22	20

In 42 of the 69 (60.9 per cent) showed metastasis, and all of these occurred frequently in the lungs but infrequently in the spleen (2 cases) and the liver (one case). Transplantation of the mammary cancer were performed on ten selected mice and

only succeeded in two cases. However, the tumors could not pass two generations.

Table 4. gives the correlation between the extent of regression-delay of the mammary glands and the mammary cancer, or mastopathy-like-changes. In the

Table 4. Correlation between the extent of regression-delay of the mammary glands and mammary cancer, mastopaty-like-changes in lactation-group-mice.

	I st group	II nd group	III rd group
Number of mice	160	21	88
Number of regression-delay	34	7	12
Per cent	21.3	33.3	13.6
Number of mastopathy-like-changes	47	7	17
Number of regression-delay	16	2	4
Per cent	34.0	28.6	23.5
Number of mammary cancer	52	4	13
Number of regression-delay	9	0	1
Per cent	17.3	0	7.7

extent of regression-delay of the mammary glands was included those females which still presented 3rd degree of regression in spite of two week's time after ablactation. The incidence of regression-delay of the mammary glands of the first group was 21.3 per cent, those of the second group was 33 3 per cent, and those of the third group was 13.6 per cent. The order of the incidence in the groups showed the same order of the incidence of mastopathy-like-changes in the groups. While mastopathy-like-changes with regression-delay occurred frequently, mammary cancer with regression-delay occurred infrequently.

The incidence of mammary cancer and mastopathy-like-changes was frequent in those which had pregnated 2, 3, 4 and 5 times. The incidence of these changes in the first and second group have pregnated more times as compared with those of the third group.

Table 5. gives the corrclation between the age and the mammary lesions. The ages were from 90 to 745 days and among them the highest incidence of abnormal lesions occurred on those over 400 days.

Table 6. gives the average age, the average cancer age of these groups and the group of virgin female mice. In the first group, mammary cancer appeared at an average age of 460 days, 556 days in the second group, and 482 days in the third group. Considerable variation was noted in the three groups; in one, the prevention of nursing had hastened the average cancer age.

The results of the virgin female group (110 mice) are given in Tables 5 and 6. The incidence of mastopathy-like-changes was 3.6 per cent (4 out of the 110 virgin females). The type were the first type (1) (3 cases) and the second type (one case). These changes appeared throughout the period from 600 to 649 days.

The third group in this study were divided into two groups (a and b). Table 7. gives these results. However, significant differences were not observed in the two groups, hence, these two groups (a and b) were included in the third group in the

Table 5. Correlation between age and mammary lesions in lactation-group-mice and in virgin female mice.

	Age in days	within 99	100 149	150 199	200 249	250 299	300 349	350 399	400 449	450 499	500 549	550 599	600 649	650 699	700 +	Totals
I st group	–	1	2	4	4		15	5	7	8	6	3	1	5		61
	Mastopathy-like changes (+)				3	6	8	2	8	3	12		3	2		47
	Mammary cancer				1		9	5	7	12	6	8	2		2	52
II nd group	–				1	3	1	1	2	1	1					10
	Mastopathy-like changes (+)				1				1	4		1				7
	Mammary cancer									2			2			4
III rd group	–		1	2	3	5	14	4	8	13	3	2	1	2		58
	Mastopathy-like changes (+)		1				2	1	1	3	5	1	1	1	1	17
	Mammary cancer					3			4	3		1		2		13
Virgin female mice	–		5	2	8	6	7	1	14	1	5	24	29		4	106
	Mastopathy-like changes (+)												4			4
	Mammary cancer															

Table 6. Average age and average cancer age in lactation-group-mice and in virgin female mice.

	I st group	II nd group	III rd group	Virgin females
Average age in days	423	422	425	490
Average cancer age in days	411	505	402	0

Table 7. The effect of the number of litters nursed by III rd group females upon the incidence of mastopathy-like-changes and mammary cancer.

	A group	B group
Number of the mice pregnated more than twice	60	23
Number of mastopathy-like-changes (Per cent)	12 (20.0)	4 (17.4)
Number of mammary cancer (Per cent)	10 (16.7)	3 (13.0)

previous experimental result. The incidence of mastopathy-like-changes and mammary cancers was found between those which had from one to five litters (20 per cent and 16.7 per cent) and those having six to 13 litters (17.4 per cent and 13.0 per cent) respectively.

2. Discussion

We have observed that the occurence of mammary cancer and mastopathy-like-changes was more frequent in those mice which were suspended from nursing (lactation) completely or incompletely compared with those with normal lactation. Hence we may surmise that the abnormal lactation may be one of the causes of mammary cancer and mastopathy in human being.

Bagg (1936) and Bagg & Jacksen (1937) reported that they have studies

regarding the influence of force breeding—rapid breeding and the prevention of nursing—upon the development of mammary cancer in mice. They reasoned that with the prevention of nursing there was retention of milk in the ducts of the mammary glands and irritating chemicals might be produced which, acting upon the epithelium, induced the development of mammary cancer.

But these results were not ratified by LITTLE & PERSONS (1940), FEKETE (1938) and later by BAGG.

MUHLBOCK and BITTNER (1948) reported that, in certain inbred strains or hybrids, rapid breeding appeared not only to increase the incidence but also to accelerate the development of the tumors; in others, especially hybrids with a low incidence, no effect was noticed.

FEKETE and GREEN (1936) reported that complete blockage of the nipples influenced the site of tumor formation in females of a cancerous strain, but did not induce mammary tumors in females of a low cancerous strain. FEKETE (1940) also obtained 9 tumors in 16 dba mice whose lactation period was prolonged to 30 days or more, as contrasted with 27 tumors in 31 mice that were permitted to nurse their offspring for only 1 day.

However, these findings were not described as mastopathy in mice. KOGURE (1946) obtained 2 mastopathy-like-changes in 47 hybrid mice whose lactation period was 30 days, as contrasted with 3 tumors and 18 mastopathys that were permitted to nurse their offspring for 5 days. In the other hand, no-mastopathys and no-tumors appeared in 51 hybrid mice of non-breeding group.

Thus, during the past forty years, studies on this subject has been made by innumerable workers, without reaching, however, any decidable conclusion; the data presented thereof were far from being uniform in spite of the similarity among the experiments.

In this investigation, we have observed that the rate of mastopathy-like-changes and mammary cancer of the group in prevention of nursing and non-breeding mice was higher as compared with those in normal breeding and suckling female mice. There can be no doubt that abnormal nursing creates a favorable situation for of the majority of mammary tumors in mice. BITTNER (1955) concluded that "if abnormal nursing exerts any influence upon the genesis of mammary cancer in mice, it would result from the increased hormonal stimulation of repeated pregnancies, and not from retention and stagnation of milk". It was summarised that the abnormities due to the abnormal nursing in the sexual rhythm should be carefully noted, as the factors influence a sensibility in the breast epithelium, possibly these abnormalities connected with an inherent predisposition to neoplastic growth.

MINAMI (1955) has studied on the changes of the levels of estrogen and progestin during lactation period in the mouse. "High levels of estrogen concentration are seen in the first and second day of lactation, though after that estrogen levels fall. In the latter half of lactation output of estrogen are ascending gradually. However, the second coming of estrus in lactation mice appears at the end of lactation and at that time abundant output of estrogen takes place suddenly. Pro-

duction of progestin is ascending gradually after parturition and the highest level of progestin is seen between 4th and 9th day of lactation."

From these hormonal changes in mice the hormonal conditions at the time of lactation-ceasing in each group may be considered as follows. In the Ist group, the estrogen showed evidently a decrease and the progesteron showed a maximum increase on the 5th day. In the IInd group, the estrogen showed an increase and the progesterone showed a little increase after parturition. Finally, in the IIIrd group the estrogen showed evidently an increase and progesterone showed evidently a decrease on the 30th day. The hormonal condition at ablactation in the IIIrd group, probably, may indicate a normal condition in normal female mice. Therefore, it is probable that a sudden change of hormonal balance, especially an increase of estrogen due to recovery of ovarial function after ablactation may stimulate the mammary gland of the Ist and IInd group. The group with high degree of endocrine imbalance showed a high rate of mastopathy and mammary cancer. From this point of view, an imbalanced endocrine stimulus action principally on the sensibility of the breast epithelium may lead to the cause of mastopathy and mammary cancer.

It seems to be that these hormonal changes at ablactation influence not only the mammary epithelium but also the other endocrine organs. CRAMER (1937) discussed the posibility of a greater susceptibility of the whole endocrine system, possibly due to a breakdown of some protective mechanism, and cited as evidence the "brown degeneration of the adrenals" observed by himself and HORNING. TAKUMA and HANEDA of our co-workers studied the endocrine organs of these mice bearing mammary cancer and mastopathy-like-changes, comparing with the normal young and old mice. They have demonstrated that there existed morphologic alterations in the endocrine organs which might be associated with the presence of mammary cancer and mastopathy-like-changes; namely, brown degeneration of the adrenal cortexs, disorder of corpora lutea and follicles of the ovaries and a relative increase of α cells and a relative decrease of γ cells in the anterior lobe of pituitary glands.

The relationship between the involution state after lactation and the incidence of mammary tumor in mice has been described by several authors. FEKETE (1938) reported that the failure to complete regression do not seem to be so dangerous from the point of view of malignant changes. However, if malignant changes of these areas do take place they probably develop slowly and later in the life of the animal. KOGURE stated that these extent of regression-delay correlated with high incidence of mastopathy-like-changes. GARDNER (1939) observed that the extent of involution of the normal mammary tissue could not be associated with the tendency to develop localized "abnormal" nodules.

In this investigation the extent of regression-delay of each group coincided with the incidence of mastopathy-like-changes was high, while those with mammary cancer was low. The reason is that the nodular mastopathy-like-changes are produced from these uninvolute state and a part of these nodules then transform into cancer. The other uninvoluted glands and nodules will gradually degenerate in a long period of time. A certain period of time is necessary for mastopathy to transform into

cancer, therefore at the outbreak of cancer it is rare to observe uninvolute state in other parts of the mammary glands.

It has been reported that the incidence rate of cancer is higher in the breeding mice compared with the virgin mice, and the reason is said to be due to the estrogen stimulation during pregnancy; its rate is various in each individual mouse. In the present experiment the incidence rate of cancer was 0, while the rate of mastopathy-like-change was as low as 3.6 per cent. Moreover, mastopathy-like-changes were observed only at the age of 600～650 days, and only 4 cases developed mastopathy.

VI. EFFECT OF ESTROGEN ON THE MAMMARY GLAND IN MICE

1. Experimental results

Sixty two female hybrid mice of 30 days old received insertions of 1.25 mg of estradiol pellet every 50 days.

1) The effects of the Ist insertion of 1.25mg of estradiol pellet.
(The changes during the period of 50 days following the initial implantation).

After 8 days the influence of estradiol on the mammary glands was observed, causing at first elongation and ramification of the mammary ducts, and the ends of mammary ducts became spindle shaped and thickened. After 50 days the elongated and branched ducts, within which secretions more or less abundant accumlate. The lobulus were not formed in this period (Fig. 50). In microscopic preparation the mammary glands consisted of distended or cystic duets filled with the concretion-like bodies were observed.

For the purpose of investigating the difference of action of the estrogen-dosis, female mice of 30 days old were inserted with 2.5 mg of estradiol pellet. Above mentioned cyst formation was observed at 10 days after the implantation of estradiol, and at about 35 days many small twigs with end-and lateral buds protruded from the elongated and branched ducts, but lobulus was minimal or absent. These mammary glands bear a resemblance to that during the earier stage of pregnancy. However, the experiment was unable to continue, because these dosis caused the death of these mice shortly after.

2) The effects of the IInd insertion. (The changes during the period from 50 to 100 days following the initial implantation)

Ninety days following the initial implantation all the mammary glands in mice alive bore a resemblance to that of the Ist insertion of 2.5 mg of estrogen. The process progressed to the formation of alveloar lobules. The Ist type (1) of mastopathy-like-changes occurred in these mammary glands. Hundred days following the initial implantation a mammary tumor appeared in one of these experimental mice.

3) The effects of the IIIrd insertion (The changes during the period from 100 to 400 days following the initial implantation).

With continued estrogenation, progressive hyperplasia and dilatation of the

ducts, increase of the connective tissues and round cell infiltration were observed (Figs. 52, 53 and 54). Namely, the mammary glands were consisted of hyperplastic areas of the Ist type (1) of mastopathy-like-changes and in places dilated fine ducts of the Ist type (2) of mastopathy-like-changes. In the epithelial proliferations in these hyperplastic areas, the appearance of cellular anomalies and monstrosities was observed more and more frequently. In this stage growth of mammary tumors was discovered in 2 cases.

When the administration of estradiol was discontinued after the IIIrd implantation, part of the mammary hyperplasia underwent gradual regression, while the other part maintained its activity. In 13 mice which survived 220 days following the initial implantation, the mammary glands changed as followes. In 4 (30.8 per cent of the 13 mice the mammary hyperplasia underwent gradual regression. In 2 (15.4 per cent) of the 13 mice some difinite areas of the same animal maintained the mammary proliferation while the majority of the other mammary hyperplasia underwent regressoin. Finally, in 7 (53.8 per cent) of the 13 mice the mammary glands maintained its activity. The results are given in Table 8.

Mammary cancer was recognized in 6 out of 44 mice which survived 91 days after the initial implantation, and metastasis in the lung was discovered in 3 of these 6 mice.

The average age and the average cancer age of estrogen group was 270 days and 225 days, respectively. Cancer appeared earier in estrogen group than in lactation group. The average age of the mice was shorter in estrogen group than in lactation group, because the estrogen tended to shorter the life of the mice.

Table 8. Incidence of mastopathy-like-changes and mammary cancer in hybrid mice receiving estradiol pellet and testosterone propionate pellet.

	Age in days		30–79	80–129	130–199	200–249	250–299	300–349	350–399	400–449	450–499	500–549	550–599	600–649	Totals
	Time after implantation in days		1–49	50–99	100–169	170–219	220–269	270–319	320–369	370–419	420–469	470–519	520–569	570–619	
	Number of implantation		1	2	3										
Estogen group	Mastopathy-like-changes	(−)	2				1	1							4
		(±)	7	12	3		1	1		1					24
		(+)		1	14	7	5		1	1					28
	Mammary cancer				1	2	1		1	1					6
Androgen group	Mastopathy-like-changes	(−)	5	4	2	3	3						2	1	20
		(±)						2	4	4	2	4			16
		(+)								2	1	1			4
	Mammary cancer														

Table 9. Details of six female mice which developed mammary cancer of the estrogen group.

	Location of tumor	Tumor age in days	Average age in days	Metastasis to
No. 521	B	130	130	(−)
No. 552	B C D	205	225	Lungs
No. 553	A B D	207	237	(−)
No. 568	D	230	360	Lungs
No. 573	A	280	280	Lungs
No. 560	A B C D	390	390	(−)

2. Discussion

It has been generally recognized that estrogen acts productively on the human and animal mammary glands. However, the effect of estrogen varied with the species and the age of the animal at which the administrations were commenced. Moreover, the effect varied with the dose, the length of treatment and type of estrogen administered. On the other hand, GARDNER (1941) reported that, with excessive doses of estrogen, the glands become stunted.

In this investigation, the observation of the mammary gland in mice treated with excessive doses of estrogen could not continue, because the mice died immediately with these doses. However, development of the glands was not interfered within these doses.

Numerous investigations have been performed on the role of estrogen in the development of the mammary glands. However, difinite conclusion has not yet been established. Branched and dilated ducts appeared in the early stage of the changes produced by slow but constant absorption of the estrogen in the mammary glands of the mice employed in the present experiment. And then, the elongated and branched fine ducts protruded from the epithelium of the duct wall. These mammary glands bore a resemblance to that during the earlier part of pregnancy. Next, the fibrous connective tissue and round cells infiltration appeared. In the secondary early stage, about 90 days following the initial implantation, the lesions in all the mammary glands of the mice employed in the present experiment appeared to resemble that of the human mastopathy so closely that it suggested that the prolonged, excessive or abnormal stimulation of the estrogen may be one of the factors accounted for the development of this disease.

TAYLOR (1933), FUJII (1938), KIMURA (1940) and other investigators reported that the effectiveness of the estrogen may be limited to the development of the duct system only and the formation of lobules attained by the stimulus of the estrogen with combined progesterone. In this investigation, it was thought that these changes might be accounted for by the effect of the estrogen at first and then by the stimulus of the corpus luteum formed by the luteotrophic hormone in the anterior pituitary. The changes of the ovaries were examined by TAKUMA of our laboratory. The essential function of the mammary glands in the reproduction of mammals depended upon a complex synchronization of these glands with the ovaries, the pituitary, the uterus and probably the adrenal cortex.

Lesions interpreted as mammary adenocarcinoma occurred in 6 mice. LOEB and SUNTZEFF (1941) reported that there was no sudden transition from quiescent ducts or acini to cancer, but a gradual development through normal growth processes. Similarity in the action of estrogens and carcinogenic hydrocarbons was further pointed out by several workers. LACASSAGNE (1936) who had reported the knowledge of the physiological properties of estrone has established "the principal action of the hormone is to provoke cellular proliferation in certain tissues, as the epithelium of the breast". However, it was summarized that endocrine imbalance resulting from prolongated estrogen effects on the mammary gland, possibly these

imbalance connected with an inherent predisposition to neoplastic growth in the breast epithelium.

After the administration of estrogen was discontinued, some of the mammary hyperplasia underwent gradual regression (30.8 per cent), while the majority of the cases continued its hyperplastic changes (69.2 per cent). In 3 (23.1 per cent) out of the latter 9 cases mammary cancer developed. Natural convalescence of mastopathy in the human being has been recognized in clinical observations, and we have also recognized the natural convalescence of mastopathy in the experimented mice.

The incidence of mammary cancer in mice showed lower percentage in the estrogen group than in the lactation groups. It was thought that difference might be accounted for the early death of the animals treated with estrogen.

How much was absorbed from this pellets? The absorption rate of the pellets in this experimental mice was 0.2 or 0.3 per cent daily. According to this account, the action of the pellets should continue a year or more, however, the stoppage of absorption in this investigation occurred after a period of about four months.

VII. EFFECT OF ANDROGEN ON THE MAMMARY GLAND IN MICE

1. Experimental results

Fourty female hybrid mice of 30 days old received insertions of 6.25mg of testosterone propionate pellet every 50 days (3 times in 150 days). The results are given in Table 8.

1) The effect of the Ist insertion of 6.25mg of testosterone propionate pellet (The changes during the period of 50 days following the initial implantation).

After 20 days the ducts became slight dilated without ramification, and also only slight dilatation of the ducts in the adipose tissue was observed in the whole mount preparations (Fig. 55). After 50 days the mammary glands became regressive. This observation corresponded exactly with that of the mice 20 days after the implantation of 12.5mg of the testosterone; that is to say when double dose is used it acted regressively from the begining (Fig. 56).

2) The effect of the IInd insertion (The changes during the period from 50 to 100 days following the initial implantation).

During the period of this stadium whole mount preparations and microscopical sections showed almost complete lack of any development in the mammary glands.

3) The effect of the IIIrd insertion (The changes during the period over 100 days following the initial implantation).

The mammary glands maintained its atrophic picture for 9 months following the initial implantation, and after that the mammary ducts gradually began to elongate and ramify (Figs. 57. 58 and 59). Many small twigs with end-and lateral buds were protruded from the elongated and branched ducts. Namely, the Ist type (1) of mastopathy-like-changes occurred in these mammary glands from 13 to 17

months following the initial implantation. Since that time, the mammary hyperplasia also underwent gradual regression. At the Table 8. indicates that all the mammary glands in 17 mice showed regression until 9 months following the initial implantation and all the mammary glands in 23 mice showed the Ist type (1) of mastopathy-like-changes (+) or (±) from 6 to 14 months following the initial implantation. Mammary cancer were not observed in the androgenized mice.

4) Five female mice, with mastopathy-like-changes of the Ist type (1) in the mammary glands 240 days following the initial implantation of 3.75mg of estradiol pellet, received insertions of 37.5 to 75mg of testosterone propionate pellet for 75 to 135 days. Fibrosis and round cell infiltration were one of the cardinal features of this experimental lesion (Fig. 60). The elongated and branched ducts were reduced in all the mammary glands of the 5 mice. However, mastopathy-like-changes of Ist type (2) appeared partially in 3 of the 5 mice (Figs. 61 and 62).

2. Discussion

The action of androgens has been reported by several authors. It is summarized that androgens are probably antagonistic to the action of the estrogens, it supresses the estrus cycle and prevents follicular maturation. In this investigation the effect of androgen on the mammary glands of immature mice varied with the dose of androgen administrated. The mammary glands in mice, treated with 6.25 mg of testosterone propionate pellet, showed at first slight dilatation but no ramification, and then, the glands underwent regression. On the other hand, the mammary glands in mice, treated with 12.5mg of testosterone propionate pellet, showed at first the state of regression.

The action of testosterone in this investigation suppresed the development of the mammary glands in female mice. However, the mammary hyperplasia appeared in mice that were discontinued of the testosterone administration between 9 to 17 months following the initial implantation.

It is probable that the action of testosterone correlated with all the endocrine system as well as the action of estrogen. It was thought that this proliferation might be accounted for by the state of relative hypoandrogenismus of the animals by these workers. Atrophy of the androgenic glands and hyperfunction of the estrogenic glands in the animals were resulted from full, continuous action of the testosterone. Takuma of our co-workers observed in these mice formation of corpora lutea and brown degeneration of the adrenal cortex from histologic examination.

Testosterone pellet were inserted into those mice with Ist type (1) (adenosis) of mastopathy-like-changes in the mammary glands. Fibrosis and round cell infiltration appeared in this experimental lesion and the mammary hyperplasia underwent general regression. However, mastopathy-like-changes of the Ist type (2) (cystic type) appeared partially in 3 of the 5 mice. It was thought that these changes might be correlated with the fibrosis. Emphasis has been placed in the fibrosis if

mastopathy in human being by BERTHELES (1913), DIETRICH (1926), SEMB (1903) and KUCKENS (1928). BERTHELES stated that "the increase in connective tissue is the primary process and that as a result of the ingrowth of interlobular tissue into the lobules the acini are separated and strangulated. Secondary to this fibrosis there develop secretion stasis, round cell infiltration and regressive and progressive epithelial changes". It was thought that mastopathy-like-changes of Ist type (2) might be also appeared by these reason.

MASUDA of our clinic reported that the Ist type and IVth type of human mastopathy (classification of Masuda) with fibrosis was no definite abnormality in average of hormone balance. On the other hand, the IInd type and IIIrd type of human mastopathy (classification of MASUDA) with epithelial proliferation showed a state of relative hyperestrogenism due to urinary hypoandrogenism. Thus, it is considered that the histological and experimental observations on the human mastopathy might be similarly accounted for by the changes of this hormonal stimulation on the mammary gland in mice.

The treatment of mammary tumors in human being has been reported by several authors. The effectiveness of androgen on mammary cancer, especially, mastopathy remained a question, and it is an important one, as to whether this excessive androgenic stimulation caused by the supression of the mammary hyperplasia from the effect of a substance which is physiologically antagonistic to estrogen or a complement for the lack of androgen in the endocrine balance. It is proper to consider that the state of dysfunction of the endocrine system in the mice may recover to when the lack of androgen is complemented for a certain period of time.

MASUDA reported that in cases of human mastopathy with hypoandrogenism androgen presented notable effect, and though in one case with normal hormonal balance androgen aggravated the lesions. Hence when treating human mastopathy with androgen one should not neglect the state of hormonal balance of the patient. The absorption rate of the pellets of the experimental mice was in average 1 per cent daily.

SUPPLEMENT. THE INCIDENCE OF TUMORS IN OTHER TISSUES

Leucaemia was present in 3 mice. Two mice belonged to the normal breeding group and one mouse treated with estrogen. The latter also combinated mammary cancer.

Uterus tumor was present in one mouse which belonged to 5 days nursing group, and had metastases in the lungs (Fig. 49). Ist type (1) of mastopathy-like-changes was observed in the mammary glands. The estrogenized females showed cystic endometrial hyperplasia and pyometra. However, uterus tumors were not observed in the estrogen group.

VIII. SUMMARY AND CONCLUSION

The relationship between the neploastic diseases of the breast and the influences of abnormal lactation and of the hormonal stimuli in mice was investigated and comparative studies with the human mastopathy have led to the following results.

1) Whole mount preparations of the mammary glands in mice were studied and compared with the sliced preparations. The physiological growth of the mammary gland of the virgin female, pregnacy, lactation and regression after lactation were studies in these hybrid mice. In the mammary glands of the multipareous females with spontaneous mammary cancer numerous abnormal changes were demonstrated. These changes were divided into 3 types of abnormal lesions by HUSEBY & BITTNER (1946) and also they were divided into 3 types of mastopathy-like-changes in mice by FUJISUE (1955). In studying the present material, these lesions were modified into 4 types by the author. In these 4 types only the Ist and the IInd type which showed aggravation.

2) In investgating the influence of nursing the experimental mice were divided into 3 groups. Each group was treated with a different method of nursing. Both the prevention of nursing and the non-breeding had effect on the incidence of mammary lesions, as compared with the normal breeding.

In the cases of mammary cancer with mastopathy-like-changes were also present in high rate.

While mastopathy-like-changes with regression-delay occurred frequently, mammary cancer with regression-delay occurred infrequently.

3) The incidence of mastopathy-like-changes in virgin female group was 3.6 per cent. The mammary cancer did not appear in this group.

4) The estrogen acted productivelly on the mammary gland in mice. At first, Ist type (1) of mastopathy-like-change occurred in the mammary glands after the implantation of estradiol pellet, and then, Ist type (2) of mastopathy-like-changes appeared occasionally in these legions. Next, mammary cancer appeared in this group. As insertions of estrogen were discontinued, the mammary glands changed as follows. The mammary hyperplasia maintained their activity (69.2 per cent). The mammary hyperplasia underwent gradual regression (30.8 per cent). In 6 out of 44 mice which survived a period of up to 91 days following the initial implantation, mammary tumors appeared.

5) The testosterone reduced the mammary hyperplasia in female mice. However, all the mammary gland in mice showed the Ist type (1) of mastopathy-like-changes from 9 to 17 months following the initial implantation. Since then, the mammary hyperplasia also underwent gradual regression. In female mice, mastopathy-like-changes of the Ist type (1) were present in the mammary glands after estradiol pellet, and they received insertions of testosterone propionate pellet. Fibrosis and round cell infiltration was one of the cardinal features of this experimental lesion and all the mammary glands reduced the proliferative lesion.

6) Mastopathy-like-changes and mammary tumors produced by estrogens and androgens, arised spontaneously from the prevention of nursing. Morphologically

similar structures were seen.

From the results of these investigations, it could be concluded as follows. The incidences of mastopathy-like-changes and mammary cancer have arised from excessive estrogenic stimulation and repeated pregnancies. These lesions appear more often in abnormal breeding and non-breeding mice than in breeding mice. The abnormalities due to abnormal nursing which cause disturbance in the sexual rhythm should be carefully noted, as these factors may influence the sensibility of the breast epithelium, and possibly these abnormalities may connect with an inherent predisposition to neoplastic growth.

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EXPLANATION OF PLATES

Fig. 3. Mammary gland of a 15 days old virgin female mouse. Whole mount preparation. × 100.

Fig. 4. Mammary gland of a 3 months old virgin female mouse. Whole mount preparation.

Fig. 5. Histological finding of Fig. 4 showing that only a few ducts with flattened and empty lumen are in adipose tissue. × 100.

Fig. 6. Mammary gland of a 16 months old virgin female mouse. Whole mount prepartion.

Fig. 7. Part of Fig. 6 under higher magnification. × 100.

Fig. 8. Histological finding of Fig. 7. × 100.

Fig. 9. Mammary gland on the 7th day of pregnancy. Whole mount preparation. × 100.

Fig. 10. Part of Fig. 9 under higher magnification. Whole mount preparation. × 100.

Fig. 11. Histological finding of Fig. 10. × 100.

Fig. 12. Mammary gland on the 20th day of pregnancy. Whole mount preparation.

Fig. 13. Part of Fig. 12 under higher magnification. × 100.

Fig. 14. Histological finding of Fig. 13 showing secretory activity. Glandular alveoli consist of one layer cells including intracellular secretion vacuoles and distended lumina filled up with secretion. × 400.

Fig. 15. Mammary gland of a mouse lactating for 5 days. Whole mount preparation. × 100.

Fig. 16. Histological finding of Fig. 15. Note the almost complete absence of the fibrous tissue. × 100.

Fig. 17. Mammary gland 5 days after lactation had stopped. Whole mount preparation. (Involution stage 5)

Fig. 18. Mammary gland 10 days after lactation had stopped. Whole mount preparation. (Involution stage 3).

Fig. 19. Histological finding of Fig. 18. × 100.

Fig. 20. Mammary gland 20 days after lactation had stopped. (Involution stage 2).

Fig. 21. Histological finding of Fig. 20. × 100.

Fig. 22. Mammary gland of a 583 days old female mouse at 350 days after the third partur-

ition. This animal belongs in the 5-day nursing group. Adipose cells greatly increased and the glandular elements reduced. Whole mount preparation. (Involution stage 1).

Fig. 23. Histological finding of Fig. 22. Mammary gland at resting stage.

Fig. 24. Areas of abnormal hyperplasia (Type 1 (1)) in whole mount preparation. Mammary gland of a 610 days old female mouse at 259 days after the third parturition. This animal belongs to the 5-day nursing group.

Fig. 25. Part of Fig. 24 under higher magnification. This nodule consists of hyperplastic fine ducts developed from small area of duct Ⅱ and duct Ⅲ. × 100.

Fig. 26. Histological finding of hyperplastic area (TypeI(1)) resembling that of adenosis in the human being. × 100.

Fig. 27. Part of Fig. 26 under higher magnification. × 400.

Fig. 28. Areas of abnormal hyperplasia (Type I(1)) showing cellular findings resembling mammary cancer. (Adenocarcinoma type A). × 100.

Fig. 29. Part of Fig. 28 under higher magnification. Tumor cells are cuboidal, containing poor chromatin nuclei. × 400.

Fig. 30. Areas of abnormal hyperplasia (Type I (2)) in whole mount preparation. In the left side an abnormal areas is seen. Mammary gland of a 488 days old female mouse at 74 days after the sixth parturition. This animal belongs to the 5-day nursing group.

Fig. 31. Part of 30 under higher magnification. The area is composed of dilated fine ducts. × 100.

Fig. 32. Histological findings of Fig. 31. × 400.

Fig. 33. Multicresentric proliferated areas of type II in whole mount preparation. Note proliferated manner radiated from a relative large ducts. Mammary gland of a 524 days old female mouse after the third parturition. This animal belongs to the 5-day nursing group.

Fig. 34. Part of Fig. 33 under higher magnification.

Fig. 35. Histological finding in an abnormal area of type Ⅱ showing that these areas consist of hyperplastic, relative large ducts and having a resemblance to that of large duct type mastopathy. The stroma was infiltrated with round cells and fibrosis. × 100.

Fig. 36. Inflammatory nodule (Type Ⅲ) in whole mount preparation.

Fig. 37. Histological finding of type Ⅲ in mastopathy-like-changes in mice. Note small hyperplastic area in extensive lymphocytic infiltration. × 100.

Fig. 38. Cystic changes of ducts (Type Ⅳ) in whole mount preparation. × 100.

Fig. 39. Histological finding of type Ⅳ in mastopathy-like-changes in mice. Note fibrosis and slight hyperplasia of the ducts throughout area. × 100.

Fig. 40. Adenocarcinoma, Type A. × 400.

Fig. 41. Adenocarcinoma, Type A. Tumor cells are acidophilic, large cytoplasm with small basal nuclei are arranged uniformly small. × 400.

Fig. 42. Adenocarcinoma, Type A. The lumina are dilated and contain a clear fluid. × 100.

Fig. 43. Part of Fig. 42 under higher magnification. A type of intracystic papillary growth. Small dark, round nuclei and large eosinophilic cells are noted. × 400.

Fig. 44. Adenocarcinoma, Type B. forming solid nests and have expansive growth. × 400.

Fig. 45. Adenocarcinoma, Type B. shows very cellular structure and frequent mitosis. × 400.

Fig. 46. Adenoacanthoma, squamous or keratinizing area from a mammary tumor. × 400.

Fig. 47. Metastasis in the lung × 100.

Fig. 48. Part of Fig. 47 under higher magnification. × 400.

Fig. 49. Fibrosarcoma (uterus). × 100.

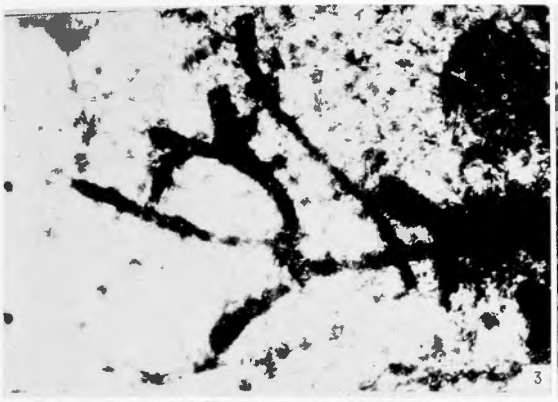
Fig. 50. Mammary gland 50 days after the beginning of implantation with estradiol -pellet of 1.25mg. Note dilated ducts with end and lateral buds. Whole mount preparation. × 100.

Fig. 51. Histological finding of Fig. 50. × 100.

Fig. 52. Mammary gland 120 days after the begining of implantation with estradiol -pellet (3 times 1.25mg). Whole mount preparation. × 100.

Fig. 53. Histological finding of Fig. 52. × 100.

Fig. 54. Mammary gland 150 days after the beginning of implantation with estradiol -pellet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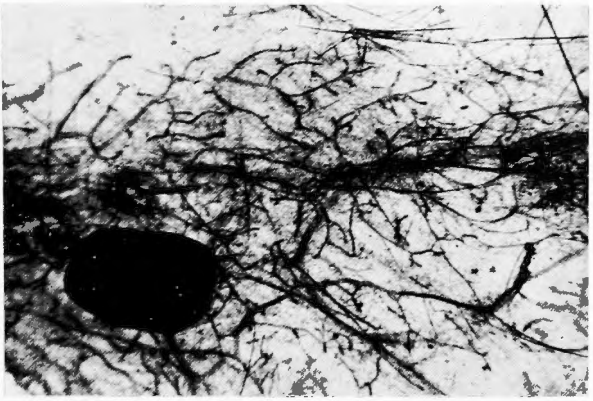

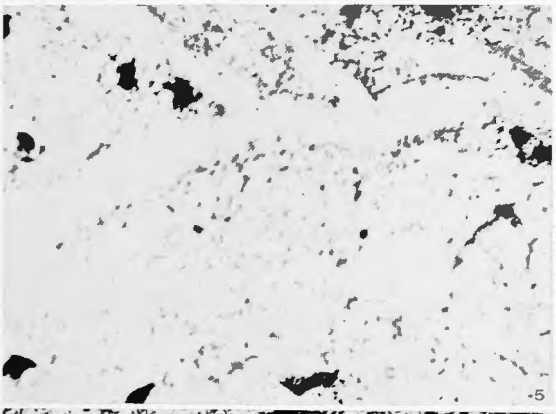
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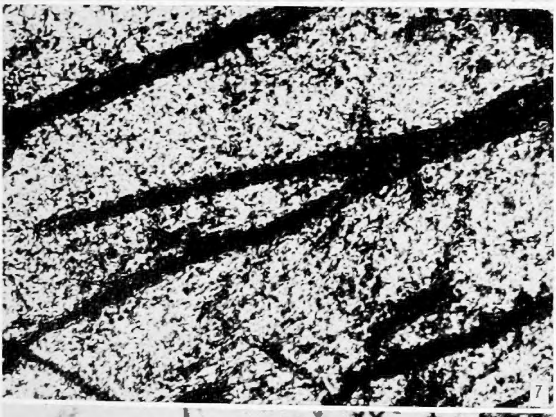
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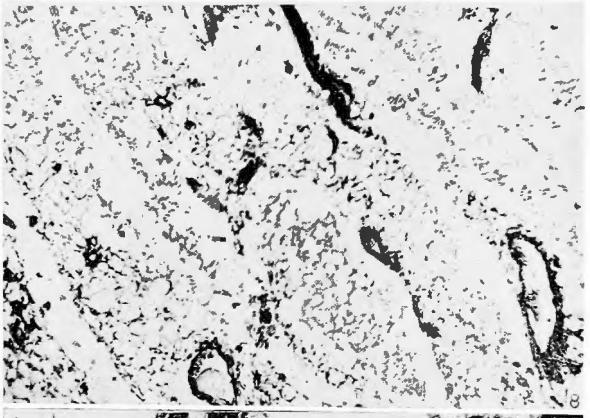
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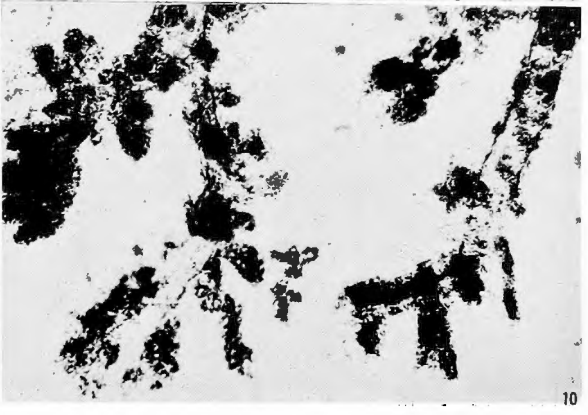
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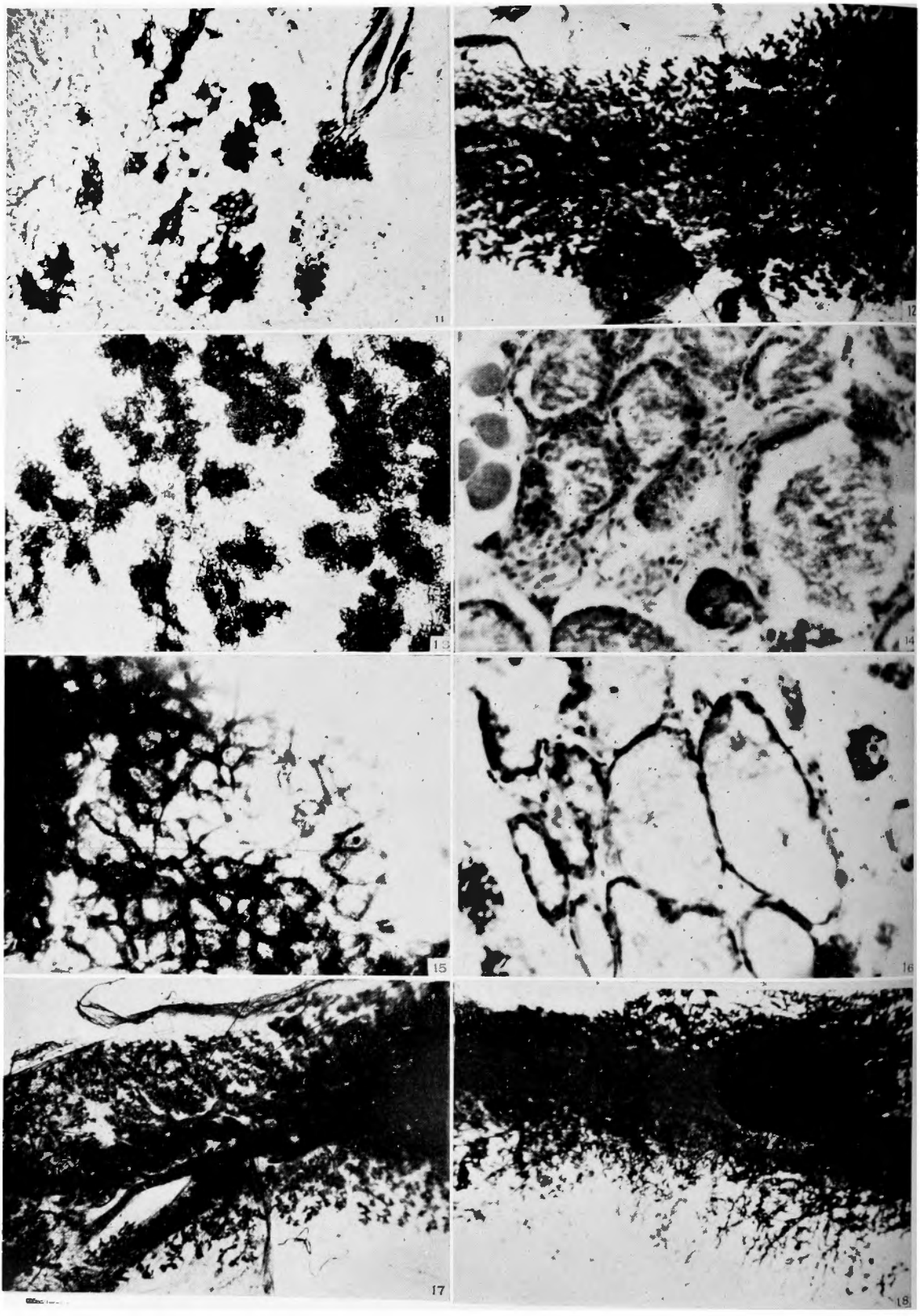
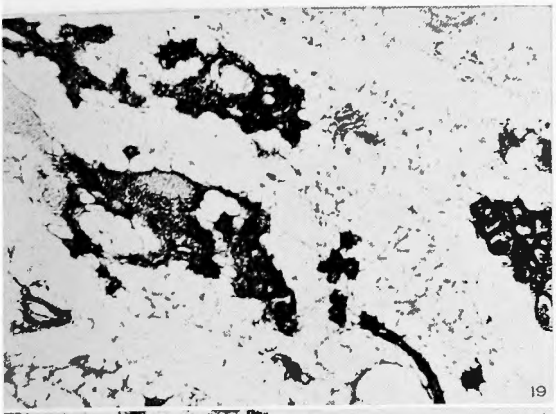


T. KOSHI Ⅱ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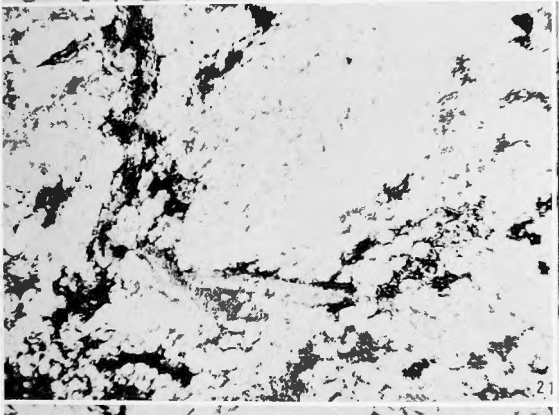
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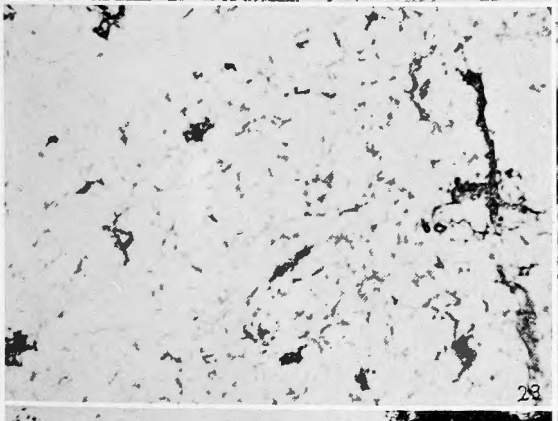
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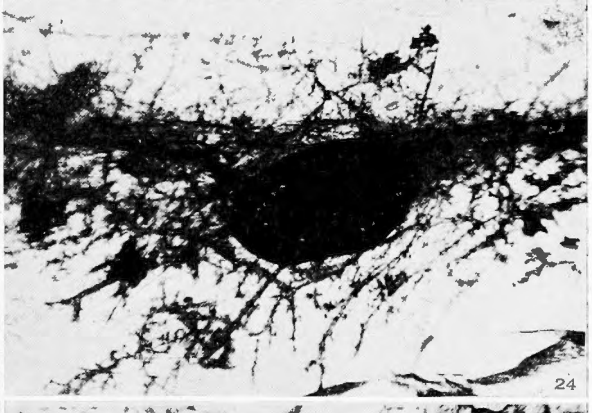
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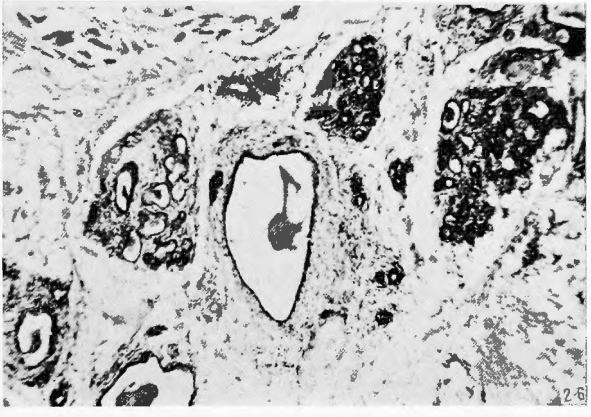
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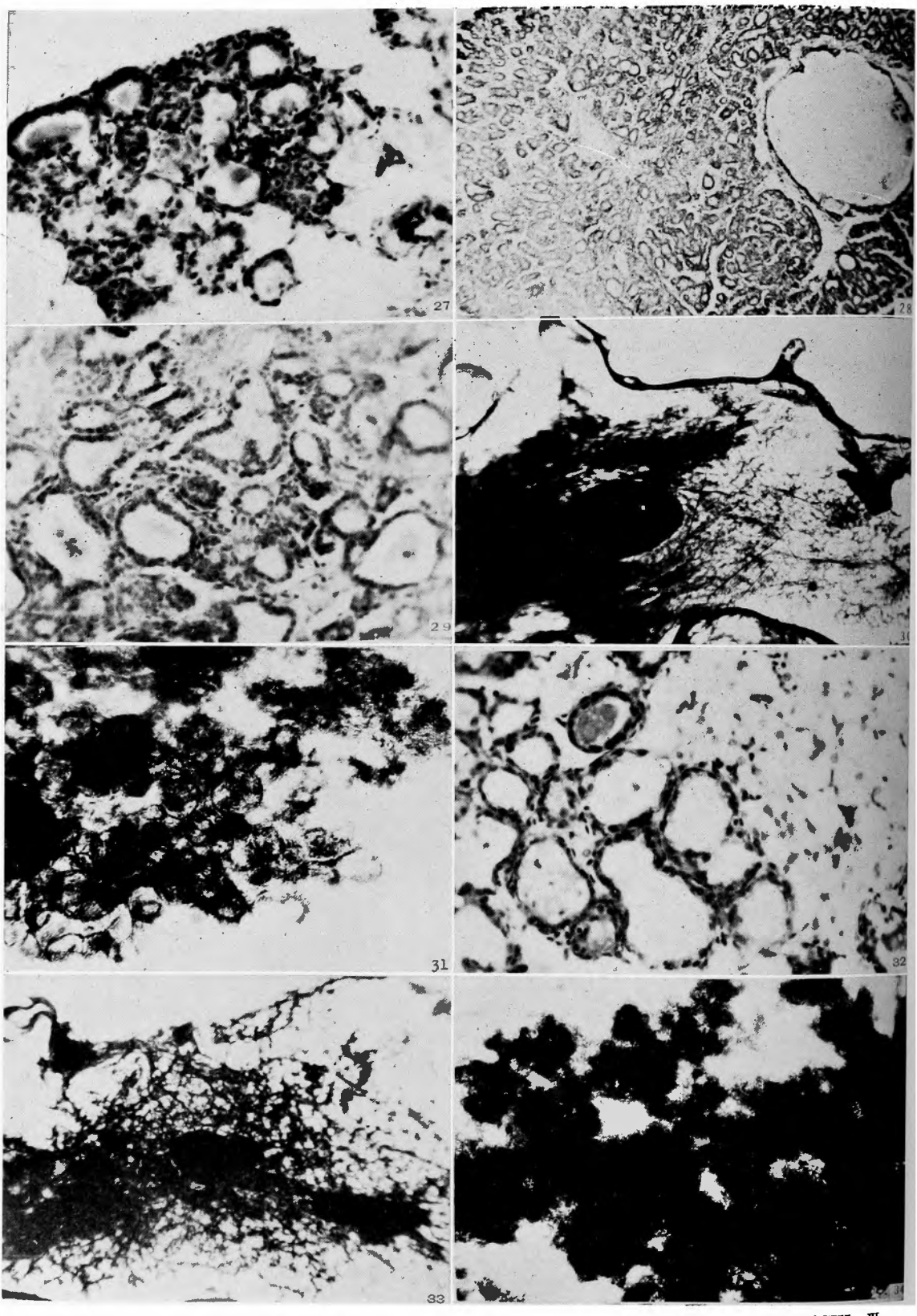

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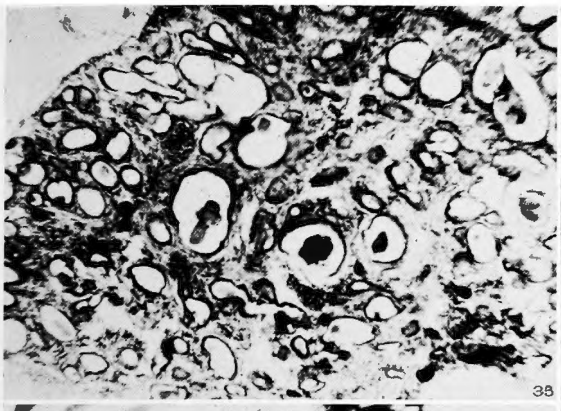
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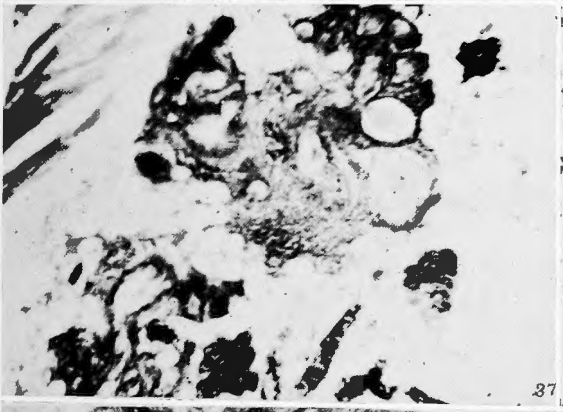
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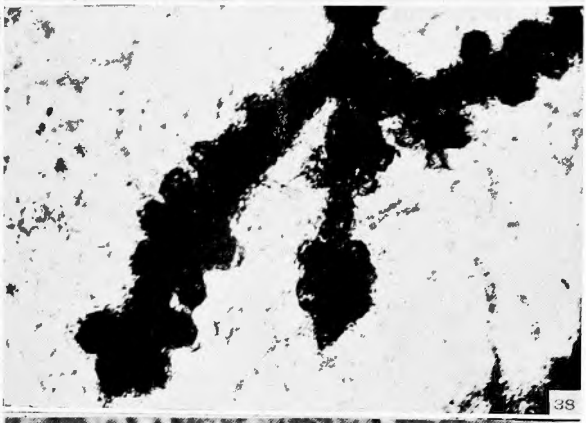
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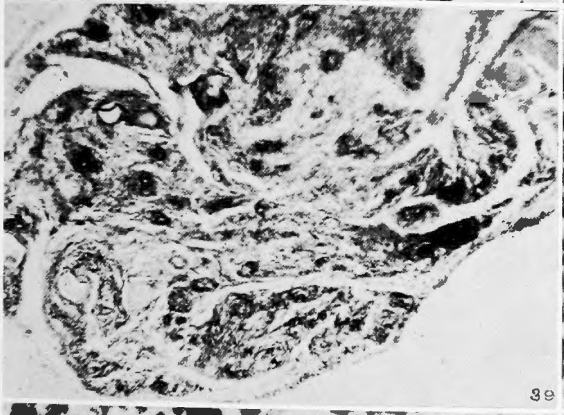
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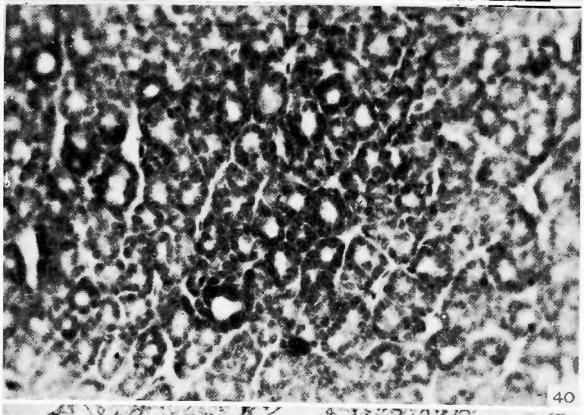
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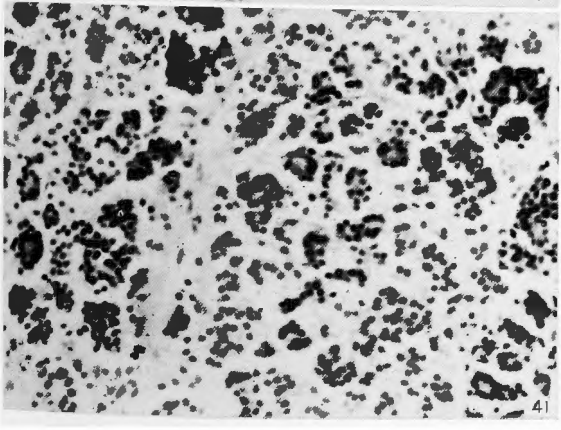
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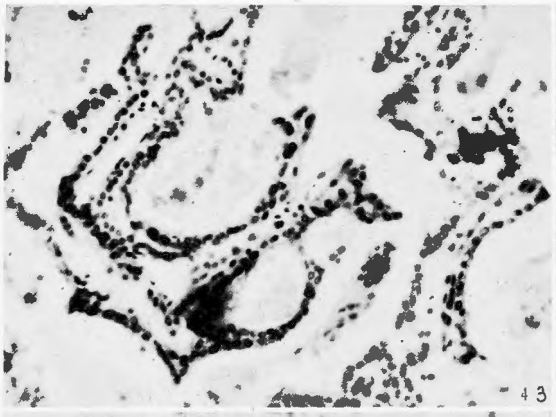
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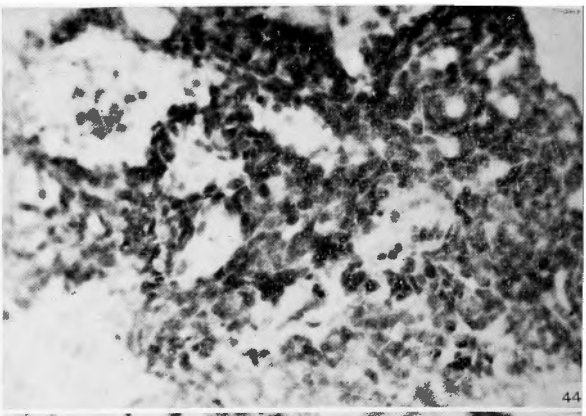
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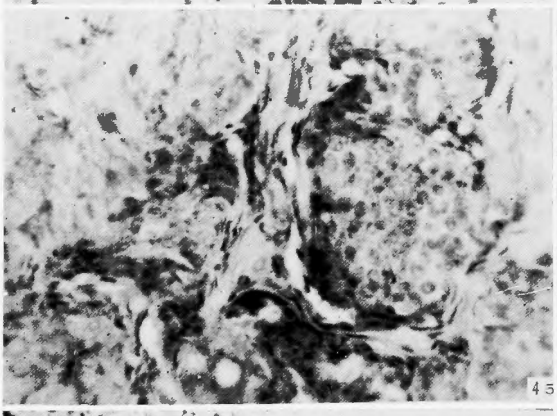
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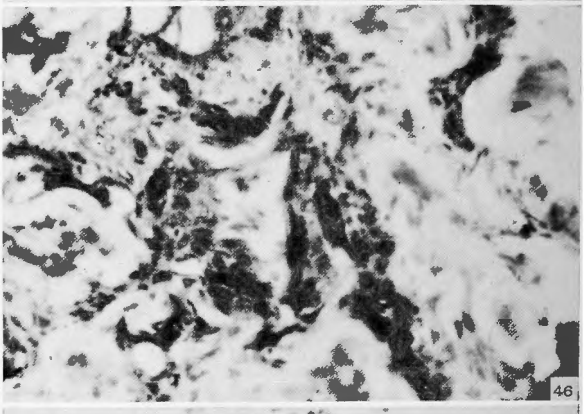
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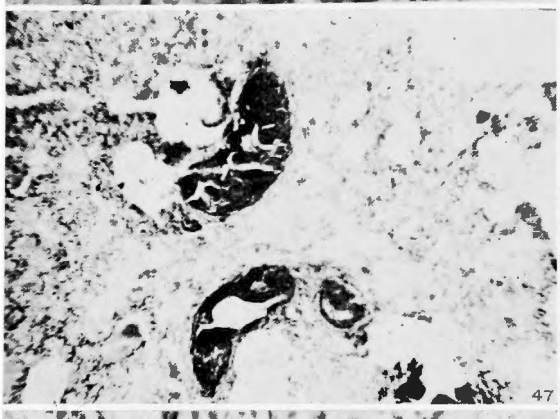
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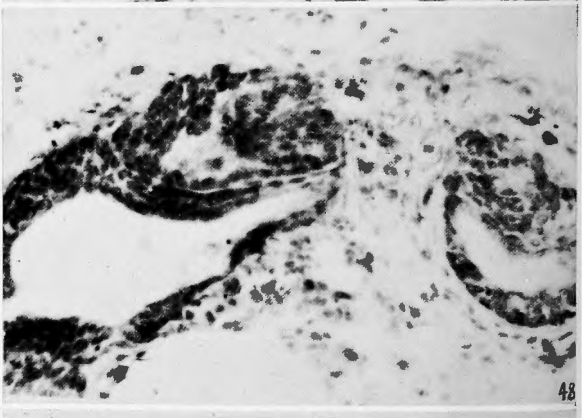
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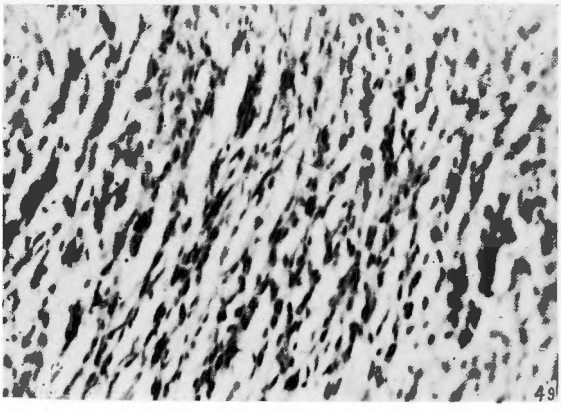
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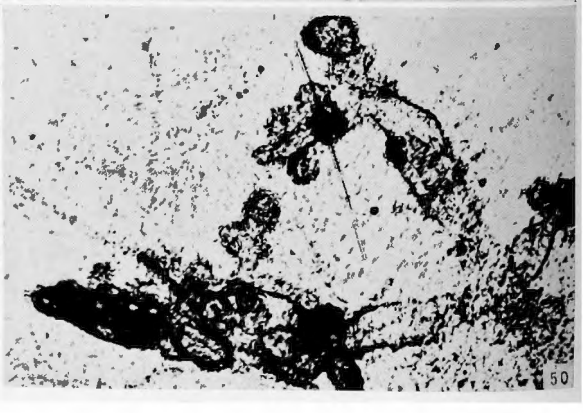
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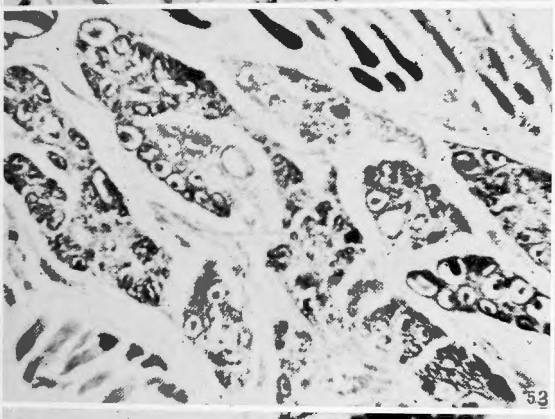
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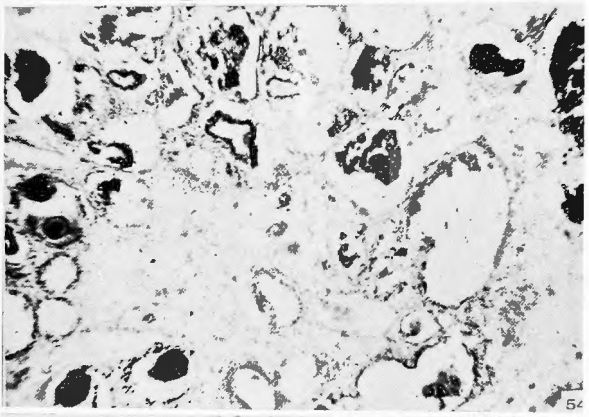
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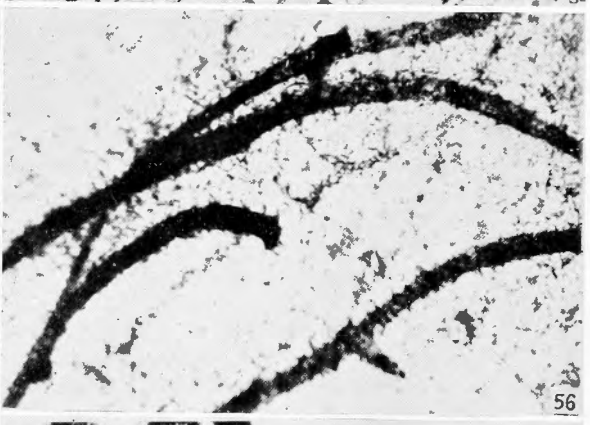
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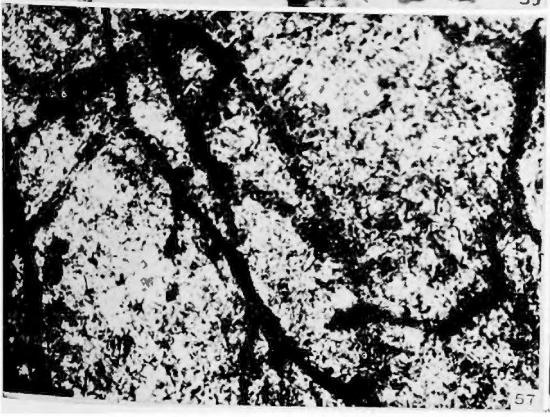
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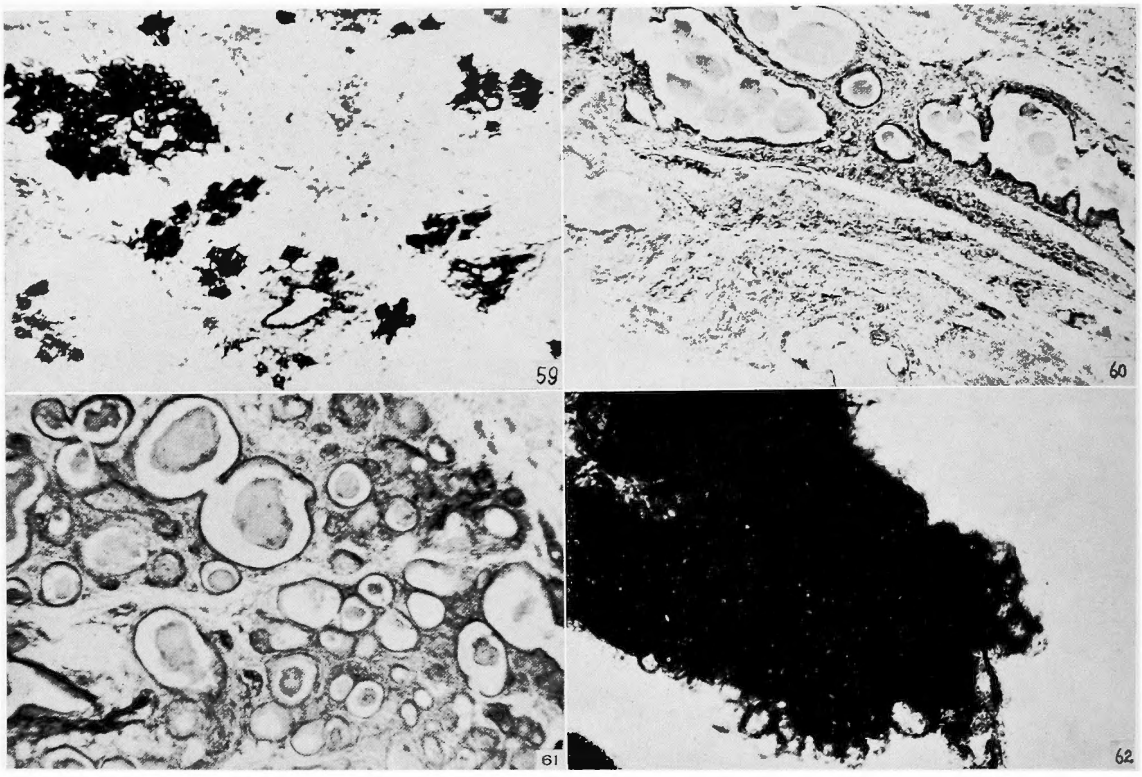

(3 times 1.25mg). Note the frequent mitoses, some atypical cells and intracystic papillary growth. × 100.

Fig. 55. Mammary gland 20 days after the beginning of implantation with testosterone-propionate pellet of 6.25mg. Ducts are slight dilatation but have no-ramification. Whole mount preparation. × 100.

Fig. 56. Mammary gland 20 days after the beginning of implanation with testosterone-propionate of 12.5mg. Note atrophic ducts. Whole mount preparation. × 100.

Fig. 57. Mammary gland 6 months after the beginning of implantation with testosterone-propionate pellet (3 times 6.25mg). Note atrophic ducts. Whole mount preparation. × 100.

Fig. 58. Mammary gland 15 months after the beginning of implantation with testosterone-propionate pellet. The nodules consist of fine branching ducts. Whole mount preparation. × 100.

Fig. 59. Histological finding of Fig. 58. × 100.

Fig. 60. The mouse with mastopathy-like-changes of the Ist type (1) in the mammary glands 240 days following the initial implantation of estradiol pellet, received insertions of testosterone-propionate pellet. Fibrosis and round cell infiltration are seen about enlarged ducts. × 100.

Fig. 61. The mouse with mastopathy-like-changes of the Ist type (1) in the mammary glands 240 days following the initial implantation of estradiol pellet, received insertions of testosterone-propionate pellet. Note the areas of mastopathy-like-changes Ist type (2). × 100.

Fig. 62. Whole mount preparation of Fig. 61.

和文抄録

乳腺腫瘍の形態発生と性ホルモンに関する実験的研究

京都大学医学部外科学教室第2講座（指導：青柳安誠教授）

大学院学生　越　哲也

乳腺腫瘍の発生と性ホルモンの関係を実験に匡す目的で岐阜産雑種マウスを使用して Estrogen 及び Androgen の投与，妊娠反復並びに授乳異常がマウス乳腺の形態学的変化及び腫瘍発生率等にいかなる影響を与えるかについて検索し，次の成績を得た．

1. マウスに於ける乳腺全体の構造を見分けうる伸展標本とその組織標本とを比較しながら実験マウス乳腺の発育，及び妊娠，授乳並に離乳等によつてそこに起る変化を観察した結果，これらの変化の中には人間の Mastopathy と比較しうる変化を見出し，さきに Huseby，藤末等の示した分類に更にマウスの Mastopathy 様変化を追加して，4型に分類した．マウスに於てかゝる変化を起す乳管は，第2，第3，特に細乳管の上皮細胞の増殖によることが多い．

2. 妊娠反復，授乳異常によつて乳腺腫瘍を発生するのは，5日間授乳の第1群，次に全然授乳をさせなかつた第2群であつて，これ等に於ては正常（30日）授乳の第3群に比べてはるかに高率に発生した．又乳癌例中に Mastopathy 様変化の共存したものを高率に認めた．離乳後に乳腺の退縮が遅延して出現したものは Mastopathy 様変化と関係を有していた．

3. 処女のまゝ一生を送らせたマウスには乳癌の発生を認めず，Mastopathy 様変化も僅かに認めたのみである．

4. Estradiol の作用で乳腺は増殖した．最初，結節状の細乳管の増殖による Mastopathy 様変化1型(1)，時には若干細乳管の嚢腫状に拡大した1型(2)も混在し，次いで乳癌の発生を認めた．Estradiol の作用を中止させると，その後もなお増殖を継続した例が69.2%で，自然治癒した例は30.8%であつた．投与開始後91日以上生存した44例中6例に，乳癌の発生を認めた．

5. Testosteron はマウスの乳腺に大体萎縮性に作用した．作用を中止させると一定時期後に一過性に増殖する時期を認めた．Estradiol で Mastopathy 様変化1型(1)の起つている時期に Testosteron を作用させると，全般的に間質結締織及び細胞の浸潤を伴うて乳腺は萎縮したが，1部乳腺の結節状の細乳管は夫々拡大して1型(2)の変化を呈した．

6. 授乳実験群とホルモン実験群の乳腺腫瘍には差異を認めなかつた．但し乳癌の発生は Estsogen 群に早期に認めた．

以上の実験結果から授乳異常が，マウス乳腺腫瘍の発生と大いに関連性がある事が考えられる．併し妊娠を反復する事で，体内の Estrogen 量が高まるということも考えられるが，われわれの実験では異常授乳法が正常授乳の場合と比較して遙かに高率であつたし，而も授乳中絶による体内ホルモンバランスの大きな変動をうけるのは，特に異常授乳法に於て著明であるからである．

即ちかゝる急激なホルモンの変動を繰り返す事によつて乳腺細胞の感受性が大なる影響を受けるものであろうと考えざるをえない．